SESSION A – NOURISHING THE BODY

Dr. Mitsugu Watanabe – INVESTIGATION OF THE BLOOD-BRAIN BARRIER PERMEABILITY OF A NOVEL ANTIOXIDANT (E6) EXTRACTED FROM SOFT BODY OF *CRASSOSTREA GIGAS*
Co-Authors: Kazuyo Fukushima, Emiko Miki, Kosuke Aritake, Yoshihiro Urade

Ms. Kazuyo Fukushima – A NOVEL ANTIOXIDANT E6 EXTRACTED FROM SOFT BODY OF *CRASSOSTREA GIGAS*: ITS LIPID ANTIOXIDANT EFFECTS IN THE BRAIN
Co-Authors: Mitsugu Watanabe, Miho Uema, Kosuke Aritake and Yoshihiro Urade

Ms. Emiko Miki - HEPATOPROTECTIVE EFFECTS OF A NOVEL ANTIOXIDANT E6 DERIVED FROM PACIFIC OYSTER (*CRASSOSTREA GIGAS*)
Co-Authors: Hirotoshi Fuda, Shu-Ping Hui, Sae joko, Hiroaki Okabe, Shigeki jin, Seiji Takeda, Takayuki Watanabe, Hitoshi Chiba, Mitsugu Watanabe

Mr. Yoshinaru Honda – EFFECTS OF SOFT BODY EXTRACTS OF *CRASSOSTREA GIGAS* ON ANTIOXIDANT ENZYMES AND ON HEPATIC ANTIOXIDANT ACTIONS
Co-Authors: Mitsugu Watanabe

Dr Kazunari Tanaka – EFFECTS OF FEEDING OYSTER PEPTIDES ON LIPID METABOLISM IN SD RATS

IN VIVO INVESTIGATION OF THE BOOD-BLAIN BARRIER PERMEABILITY OF A NOVEL ANTIOXIDANT (E6) EXTRACTED FROM SOFT CRASSOSTREA GIGAS

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[Abstract]
We discovered an antioxidant (3,5-dihydroxy-4-methoxybenzyl alcohol; E6) present in the soft body of pacific oysters and subsequently synthesized and characterized this compound. E6 has a low molecular weight (170 g/mol) and was postulated to possess blood-brain barrier (BBB) permeability due to its amphipathic nature, possessing both hydrophilic and hydrophobic properties. We therefore investigated its BBB permeability.

[Methods]
In 9-week-old male C57BL/6NCrslc mice, E6 dissolved in saline was orally administered at a dose of 300 mg/kg in 0.2 mL/20 g body weight. Blood and whole brain samples were collected before oral E6 administration (0 hour), 10, 30 minutes, 1, 2 hours, and 6 hours post-administration. E6 levels were analyzed using LC-MS/MS in the processed samples.

[Results]
E6 was not detected in pre-administration mouse plasma and brain, suggesting that E6 is not physiologically present in mice. E6 was detected using LC-MS/MS in the brain sample collected 10 minutes after oral E6 administration, demonstrating the BBB permeability of E6. E6 half-life was 12.4 minutes in the plasma and 28.9 minutes in the brain. E6 concentration was greater in the brain than plasma from 30 minutes after administration. Furthermore, E6 was detected in the brain, but not in the plasma, at 180 minutes after administration.

[Conclusion]
This study suggested E6 isn’t present in mouse plasma and brain. Moreover, the results demonstrated the *in vivo* BBB permeability of E6 and showed its retention time is longer in brain than in plasma, suggesting its efficacy as an antioxidant in alleviating oxidative stress in brain.

A NOVEL ANTIOXIDANT (E6) EXTRACTED FROM SOFT BODY OF *CRASSOSTERA GIGAS*: ITS ANTIOXIDANT EFFECTS IN THE BRAIN

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[Objective] An antioxidant that can penetrate into the blood-brain barrier (BBB) is desirable to counteract oxidative stress in the brain.
We characterized 3,5-dihydroxy-4-methoxybenzyl alcohol (E6), a novel amphipathic antioxidant derived from the soft body of *Crassostera gigas*, and assessed its BBB permeability. In the present study, we investigated the anti-oxidative capacity of E6 in the brain.

[Methods] Twenty-four 9-week-old male KK-A^y/TaJcl mice were divided into three groups (control, E6 and vitamin E (VE)). The control group received a 0.5% methylcellulose solution (MC), the E6 group received 79 mg/kg E6 in MC and the VE group received 200 mg/kg VE (equimolar to E6) in MC. All animals were given the respective solutions via oral gavage for 14 days. Brain TBARS levels were measured.

[Results and Discussion] The brain TBARS level was significantly lower in the E6 group (32.49±1.25 μM/g wet tissue) compared to control group (39.17±1.77), indicating a lipid antioxidant property of E6. In contrast, the brain TBARS level was not decreased in the VE group. These findings indicate that, in contrast to VE, which has a long half-life (16 hours) and accumulates in the brain but tends to be converted to a radical molecule, E6 has a short half-life (28.9 minutes) and is eliminated rapidly from the brain after exhibiting its antioxidant actions.

[Conclusion] The BBB permeability of E6 and its lipid antioxidant effect in the brain were observed *in vivo*, and it was postulated that the antioxidant capacity of E6 is derived from its amphipathic nature and short half-life.

HEPATOPROTECTIVE EFFECTS OF A NOVEL ANTIOXIDANT E6 DERIVED FROM PACIFIC OYSTER(*CRASSOSTREA GIGAS*)

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[Background and Purpose] Although antioxidants and omega-3 fatty acids are used in the treatment of diseases related to oxidative stress, a more effective hepatoprotective antioxidant is desired. We identified a new antioxidant (3,5-dihydroxy-4-metoxybenzyl alcohol; E6) derived from oyster extracts (Food Chem., 2012).

In order to investigate the potential hepatoprotective effects of E6, in the present study, After the addition of Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and E6,we measured the activity of transaminases as markers for liver function test (AST and ALT) in cell culture media using a human liver-derived cell line (C3A). In addition, genes related to liver fibrosis were quantified after the addition of E6 to C3A cells.

[Methods and Results] The transaminase activity in the cell culture media was quantified. Although the transaminase activity decreased dose-dependently in the E6-treated group, no such decrease was observed in cells treated with DHA, EPA. Moreover, in E6-treated C3A cells, real-time PCR quantification of liver-related genes revealed a significant downregulation of genes that are usually upregulated during fibrosis (αSMA, TGFβ, TNFα), and a significant upregulation of a gene that suppresses fibrosis (MMP-1).

[Discussion] C3A cells treated with E6 exhibited a statistically significant a decrease in transaminase activity in the cell culture media, downregulation of genes that increase during fibrosis, and upregulation of a gene that suppresses fibrosis. Based on these results, it is likely that E6 exhibits not only oxidation-suppressing effects, but also cell-protective properties in human hepatocytes.

EFFECTS OF SOFT BODY EXTRACTS OF CRASSOSTREA GIGAS ON ANTIOXIDANT ENXYMES AND ON HEPATIC ANTIOXIDANT ACTIONS

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[Introduction] In this study, we investigated the effects of oyster meat extracts on hepatic antioxidant enzyme activities and antioxidant actions in a diabetic mouse model with persistent oxidative stress.

[Methods] Sixteen 9-week-old KK-Ay/Ta Jcl mice were divided into a control group and an extract group. The control group administered a 0.5% methylcellulose (MC) solution and the extract group administered an extract solution formulated with 67.5 mg of oyster meat extract suspended in 10 mL of 0.5% MC. All animals were given the respective solutions at a dose of 0.8 mL/40 g body weight once daily for 14 days via oral gavage. After administration, the liver SOD, GSH-Px activity, 8-OHdG and TBARS concentration were measured in these two groups.

[Results] SOD activity was significantly greater ($p < 0.01$) in the extract group (4.69±0.49 U/mg wet tissue) compared to the control group (2.59±0.47 U/mg), demonstrating the SOD-activating effects of the extracts. Also, GSH-Px-activating effect was observed in the extract group.
8-OHdG concentration was lower ($p <0.05$) in the extract group (19.16±2.38 ng/g wet tissue) compared to the control group (28.07±2.03 ng/g), demonstrating the DNA antioxidant effects of the extracts in the liver. Also, TBARS concentrations were significantly lower in the extract group compared to the control group, showing lipid antioxidant effects of the extracts in the liver.

[Conclusion] We observed DNA and lipid antioxidant effects that are considered to result from the elevated activities of SOD and GSH-Px induced by the administration of oyster extract.

EFFECTS OF FEEDING OYSTER PEPTIDES ON LIPID METABOLISM IN SD RATS

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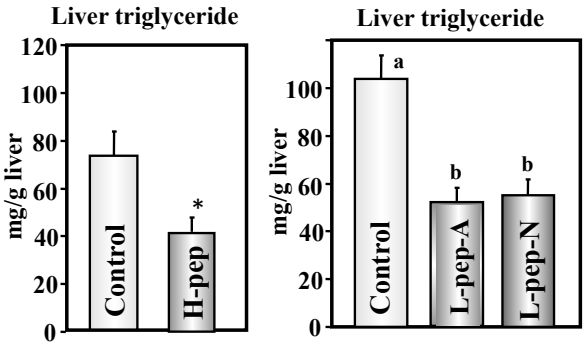
Oyster contains a relatively large amount of glycogen, protein, and minerals such as calcium and zinc. Oyster is also rich in lipid components, EPA, DHA and non-cholesterol sterols. Authors previously showed that feeding oyster induced the reduction of serum and liver cholesterol and triglyceride concentrations. In addition to the lipid and non-lipid components, It is possible from our previous results that oyster protein induces hypolipidemic activity. In the present study, the effect of oyster peptides obtained by the hydrolysis of oyster protein on lipid metabolism was investigated. High molecular peptides (molecular weight: 7,100 ~ 53,400) were prepared by hydrolyzing oyster protein with 2 proteases. SD rats were fed a diet containing 15% casein and 5% high molecular peptide as the protein source for 3 weeks. Oyster peptides exerted a significant reduction in serum cholesterol and hepatic triglyceride concentrations. Next, two types of low molecular peptides were obtained by some proteases, and more than 40% of peptides were molecular weight of less than 500. Feeding the diet containing 15% casein and 5% low molecular peptide and 0.5% cholesterol for 4 weeks decreased serum and liver triglyceride levels in SD rats. The present study revealed that oyster peptide improves lipid metabolism, and it is promising in developing a novel functional food for human health.



Effects of feeding oyster peptides on liver triglyceride concentrations.
H-pep: High molecular peptide
*: Significant differ ence from the control group at $p < 0.05$.
Means ± SE (n = 6)
L-pep-A: Low molecular peptide type A
L-pep-N: Lo w molecular peptide type N
^{ab}: Different letters show significant difference at $p < 0.05$. Means ± SE (n = 6)